Result:

1. We tested protein expression in E.coli at different temperatures and found that optimum temperature is 25℃.
2. Expression of XynB with our method was high and stable, expression of ArfB and ManA1 was not so prominent, but detectable with SDS-PAGE and commassie bright blue staining method.
3. Two types of competent cells, BL21(DE3) and BL21(DE3 CodonPlus) exhibit similar productivity to XynB, but expression of ArfB and ManA1 are only detectable when expressed in BL21(DE3 CodonPlus)

Procedure:

1. Transform formerly constructed and verified plasmids, designated pET22b(+)_ArfB, pET22b(+)_ManA1, pET22b(+)_x1 to BL21(DE3 CodonPlus), transform pET22b(+)_x2 to BL21(DE3)
2. Spread transformed bacteria to ampicillin loaded LB plate, culture at 37℃ overnight
3. Inoculate several colonies of each plate to LB medium, perform colony PCR for each culture(Figure 1)

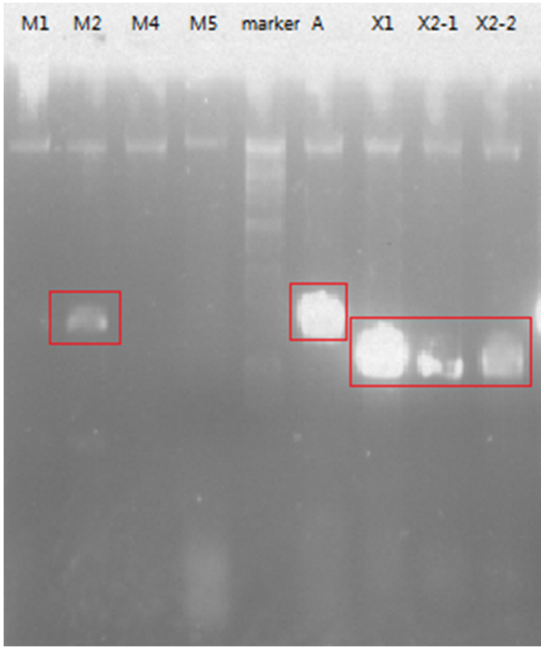

Figure1, M2, A, X1, X2-1, X2-2 are positive.

4. Continue to breed each colony PCR verified cultures in ampicillin loaded LB medium as following procedure:

For large amount of XynB production, we used this formerly testified method	add about 2 ml X2-2 to each of 300 ml sterilized ampicillin loaded LB medium culture at 37℃ for 4h, measure OD 600 = 1±0.2 add IPTG(1M) 120μl to each flask

	culture at 25℃ for 7h centrifuge all 900mL culture to get 1.50 g bacteria pellet resuspend pellet with 15ml osmotic shock buffer A(hypertonic) store at 4℃ overnight(approximately 10h) centrifuge all resuspention mixture, collect supernatant as X2-2 suc resuspend pellet with 20ml osmotic shock buffer B(hypotonic) store at 4℃ for 1.5h centrifuge, collect supernatant as X2-2 sup resuspend pellet with 10ml H2O and dilute for 5 times as X2-2 S
To test protein expression level at 18℃, we conducted this experiment A,M2,X1,X2-1(18℃, ① group)	Retrieve all bacteria cultures from 4℃ freezer (each 5 ml), add all 5 ml to each of 10 ml sterilized ampicillin loaded LB medium culture at 37℃ for 4h, measure OD 600 = 2 dilute to OD 600 = 1, volume of each tube is 15ml add IPTG(1M) 12μl to each flask culture at 18℃ for 19h centrifuge all 30mL culture to get 0.3g-0.4g bacteria pellet resuspend pellet with 1ml osmotic shock buffer A(hypertonic) store at 4℃for 1.5h centrifuge all resuspention mixture, collect supernatant as A①suc, M2①suc, X1①suc, X2-1①suc, respectively resuspend pellet with 1ml osmotic shock buffer B(hypotonic) store at 4℃ for 1.5h centrifuge, collect supernatant as A①sup, M2①sup, X1①sup, X2-1①sup, respectively resuspend pellet with 1ml H2O as A①s, M2①s, X1①s, X2-1①s, respectively
To test protein expression level at 25℃, we conducted this experiment A,M2,X1,X2-1(25℃, ② group)	Retrieve all bacteria cultures from 4℃ freezer (each 5 ml), add all 5 ml to each of 10 ml sterilized ampicillin loaded LB medium culture at 37℃ for 4h, measure OD 600 = 2 dilute to OD 600 = 1, volume of each tube is 30ml add IPTG(1M) 12μl to each flask

	culture at 25℃ for 7h centrifuge all 30mL culture to get 0.7g-0.8g bacteria pellet resuspend pellet with 1ml osmotic shock buffer A(hypertonic) store at 4℃ overnight(approximately 10h) centrifuge all resuspention mixture, collect supernatant as A②suc, M2②suc, X1②suc, X2-1②suc, respectively resuspend pellet with 1ml osmotic shock buffer B(hypotonic) store at 4℃ for 1.5h centrifuge, collect supernatant as A②sup, M2②sup, X1②sup, X2-1②sup, respectively resuspend pellet with 1ml H2O as A②s, M2②s, X1②s, X2-1②s, respectively
To test protein expression level at 28℃, we conducted this experiment A,M2,X1,X2-1(28℃, ③group)	Retrieve all bacteria cultures from 4℃ freezer (each 5 ml), add all 5 ml to each of 10 ml sterilized ampicillin loaded LB medium culture at 37℃ for 4h, measure OD 600 = 2 dilute to OD 600 = 1, volume of each tube is 30ml add IPTG(1M) 12μl to each flask culture at 28℃ for 7h centrifuge all 30mL culture to get 0.7g-0.8g bacteria pellet resuspend pellet with 1ml osmotic shock buffer A(hypertonic) store at 4℃ overnight(approximately 10h) centrifuge all resuspention mixture, collect supernatant as A③suc, M2③suc, X1③suc, X2-1③suc, respectively resuspend pellet with 1ml osmotic shock buffer B(hypotonic) store at 4℃ for 1.5h centrifuge, collect supernatant as A③sup, M2③sup, X1③sup, X2-1③sup, respectively resuspend pellet with 1ml H2O as A③s, M2③s, X1③s, X2-1③s, respectively

SDS-PAGE

Add 5μl 3*loading buffer to 10μl of each sample to prepare loading sample

Load 15μl of each loading sample to 1.5mm thick SDS-PAGE with 10%separate gel

Use transformed, before IPTG treatment culture as negative controls

Molecular weight of proteins:

ArfB 55985.8116600001

XynB 44522.7031800001

ManA1 58112.0507300001

Stain with commassie bright blue R250 solution, destain with destaining agent(45% methanol, 45% H_2O, 10% acetic acid) to detect protein expression(Figure 2)

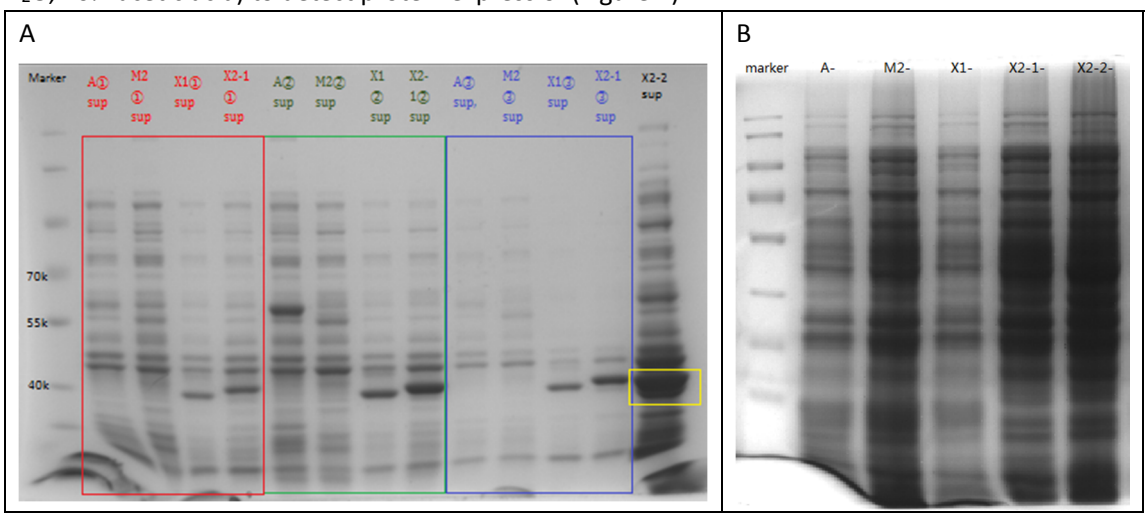

Figure 2, (A)ArfB and ManA1 yield maximized when cultured at 25℃ after adding IPTG, X2-2 sup shows prominent XynB yield. (B)negative controls show very little leaky expression.